AD NUMBER

AD870370

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bdrl, d/a, ltr, 29 sep 1971

AD

TECHNICAL MANUSCRIPT 610

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Frederick B. Abeles
Lyle E. Craker
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JUNE 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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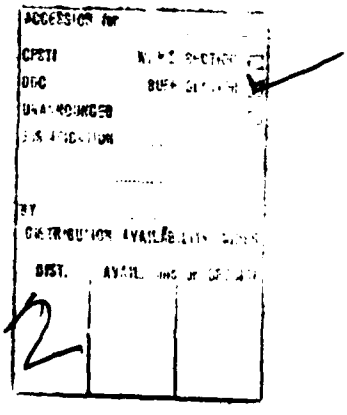

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ABSCISSION: THE PHYTOGERONTOLOGICAL EFFECTS OF ETHYLENE

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Plant Physiology Division
PLANT SCIENCES LABORATORIES

Project 1B562602AD04

June 1970

ABSTRACT

The role of ethylene in the aging of bean (*Phaseolus vulgaris* L. cv. Red Kidney) petiole abscission zone explants was examined. The data indicate that ethylene does accelerate aging in addition to inducing changes in break strength. Application of ethylene during the aging stage (stage 1) promoted abscission when followed by a second ethylene treatment during the cell-separating stage (stage 2). The half-maximal effective concentration of ethylene to induce aging was about 0.3 ppm; 10 ppm was a saturating dose. CO_2 reversal of ethylene action during stage 1 was incomplete and gave ambiguous results. CO_2 (10%) reversed the effect of 10 ppm ethylene but not that of 1 ppm ethylene. The possibility that ethylene not only accelerated aging but that it was also a requirement for it was also tested and experimental evidence in favor of this idea was obtained. It was concluded that ethylene plays a dual role in the abscission of bean petiole explants: a phytogerontological effect and a cellulase-inducing effect.

I. INTRODUCTION*

The concept that abscission of explants occurs in three stages was developed to organize experimental data into a meaningful sequence and to serve as a framework for experimental design. Stage 1 can be thought of as an aging period, stage 2 as the interval when cell-wall-degrading enzymes, such as cellulase, are induced, and stage 3, the period in which these enzymes act, causing a loss of break strength in the region of cell separation.[1] These three stages are a refinement of the original two-stage concept advanced by Rubinstein and Leopold.[2] They reported that auxin could either block (stage 1) or stimulate (stage 2) abscission, depending on the time the hormone was added to abscission zone explants. Abeles and Rubinstein[3] subsequently reported that the stimulatory action of auxin was actually due to its ability to increase ethylene production from explants and that ethylene would promote abscission only when it was applied during stage 2. The idea that the role of ethylene in abscission was limited to stage 2 explants was strengthened by the observation that ethylene had no effect on protein or cellulase synthesis during stage 1.[4,5]

Additional support for the idea that stage 1 was associated with aging and stage 2 with ethylene action came from the experiments by Abeles, Holm, and Gahagan.[6] Auxin and cytokinin delayed both senescence (measured as a loss of chlorophyll, protein, and RNA) and abscission. Ethylene, on the other hand, had no effect on chlorophyll and RNA breakdown and only a small effect on protein degradation when applied during stage 1. Dela Fuente and Leopold[7] confirmed that ethylene did not have a detectable effect on the mobilization of materials from the pulvinus.

However, two observations suggest that the idea that ethylene has no effect on aging or stage 1 is wrong. First, Burg[8] pointed out that it would be difficult to observe an effect of ethylene on stage 1 because of the high rate of ethylene evolution associated with freshly excised tissue.

Secondly, it is a fairly common observation that ethylene causes leaves to turn yellow and abscise. On this basis, Hallaway and Osborne[9] make the reasonable suggestion that ethylene is an aging hormone and that the defoliating action of ethylene is due to its ability to accelerate senescence in plants.

We believe that the experiments described here indicate merit in both points of view. It is true that explants must pass through an aging period for ethylene to promote cellulase synthesis, which causes the resulting loss of break strength. However, it is also true that ethylene accelerates aging in explants so that it now appears that the gas plays a dual role in abscission.

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II. MATERIALS AND METHODS

A. PLANT MATERIALS

The methods used to grow bean (Phaseolus vulgaris L. cv. Red Kidney) plants and to prepare and store explants have been described earlier.[8,10,11]

B. APPLICATION OF CHEMICALS

Earlier papers from this laboratory have described the treatment of explants with ethylene and CO_2 in gas collection bottles,[4] the measurement of ethylene by gas chromatography,[12] the injection of solutions, in these experiments 1 µliter of 10^{-3} M indole-3-acetic acid (IAA), into the separation layers of explants,[11] and break strength measurements by a recording abscissor.[13] When ethylene was applied in a gas stream the method of Pratt et al.[14] was used, except for the experiment shown in Table 2. In that case, 1 ppm ethylene from a compressed air cylinder was mixed with an air stream to give 0.1 and 0.01 ppm ethylene. To prevent desiccation of plant material the gas streams were humidified by bubbling them through water.

Most of the results are presented as percentage initial break strength at some fixed time, usually 24 hours. This means that the average break strength of a set of 10 explants 24 hours old was subtracted from the average break strength (between 350 and 400 g) of 10 freshly excised explants, and the difference was divided by the initial break strength and multiplied by 100. Experimental points shown in figures and tables are the averages of three or more sets of 10 explants.

III. RESULTS

Figure 1 shows that the earlier 10 ppm ethylene is added to explants, the greater its subsequent effect on break strength as measured at 24 hours.

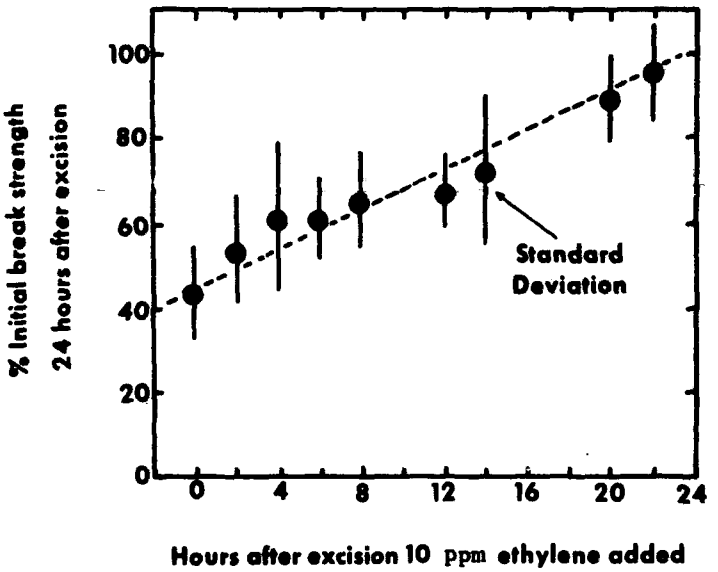

FIGURE 1. Effect of Adding 10 ppm Ethylene to Explants at Various Times after Excision. Ethylene was injected into gas collection bottles at the times indicated. Explants were aerated until the ethylene treatment.

Figure 2 shows the effect of various ethylene treatments during a 24-hour period after excision. The left side of the figure indicates the duration of either ethylene or air treatments; the right side shows the effect of these gas treatments on the break strength measured at 24 hours. The results indicate the ethylene treatments during the first 6 hours are without effect unless they are followed by a second exposure to the gas. Break strength at 24 hours was unaffected when explants were treated with 10 ppm ethylene for 2, 4, or 6 hours during stage 1 and no ethylene was added during stage 2 (see upper four bars of figure). However, if the explants were treated with 10 ppm ethylene during stage 2, then 2, 4, or 6 hours of ethylene during stage 1 caused correspondingly greater loss of break strength (see lower five bars of figure).

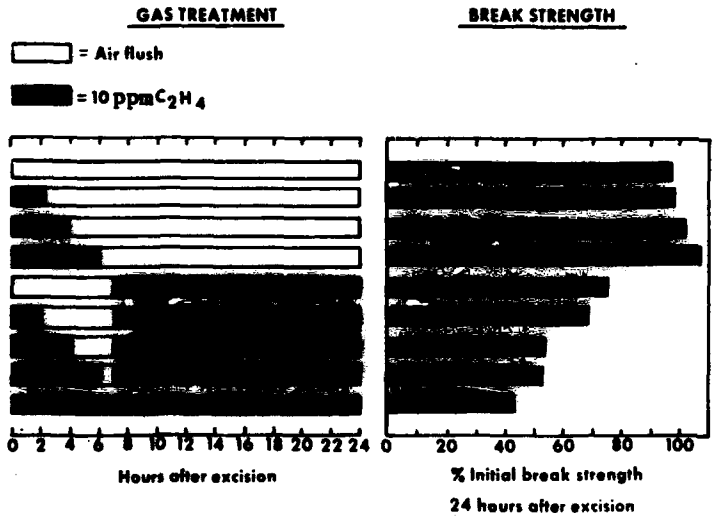

FIGURE 2. Effect of Ethylene on Stage 1. The left side of the figure indicates the periods of 10-ppm ethylene treatment by solid bars. The gas was added by injection into sealed gas collection bottles. The open bars represent the length of time the bottles were flushed with air. The graph on the right side shows the effects of these treatments on break strength.

The effect of ethylene on aging can also be demonstrated by measuring the length of time IAA retains its ability to prevent a loss of break strength. In these experiments, the explants were treated with IAA at specified times after excision, and the ability of auxin to block abscission was measured at 24 hours. Figure 3 shows that 12 hours elapsed before IAA lost its effectiveness in control explants, and ethylene reduced this period to 8 hours..

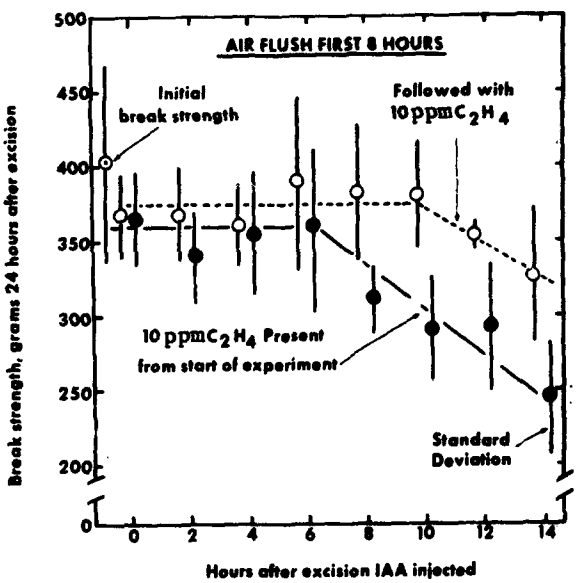

FIGURE 3. The Shortening of Stage 1 by Ethylene Measured by Loss of the Ability of IAA to Block Abscission. IAA was injected into explants at the times indicated. One set of explants was exposed to 10 ppm ethylene from the start of the experiment; the second set was flushed with air for the first 8 hours, followed by sealing the bottles and injecting 10 ppm ethylene into the gas phase.

Figure 4 is a dose-response curve for the effect of ethylene on aging. In these experiments, explants were exposed to various concentrations of ethylene during the first 6 hours after excision followed by 10 ppm for the next 18 hours. The results indicate that 0.3 ppm gave a half-maximal response, but that 10 ppm is a saturating dose.

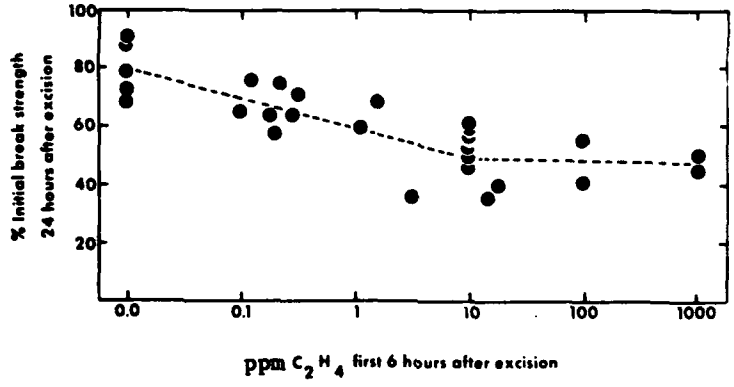

FIGURE 4. Dose-Response Curve for the Effect of Ethylene on Stage 1. Explants were flushed with the indicated ethylene concentrations for the first 6 hours after excision. They were then sealed and 10 ppm ethylene was added to the gas phase.

If the active site for ethylene on the aging process is analogous to sites for fruit ripening, growth, and stage 2 abscission, then CO_2 should be able to block the effectiveness of ethylene. The data in Table 1 show that 10% CO_2 partially blocked the aging effect of 10 ppm ethylene. However, CO_2 did not reverse the effect of 1 ppm ethylene.

TABLE 1. CO_2 REVERSAL OF THE ETHYLENE EFFECT ON STAGE 1[a/]

Treatment During First 6 Hours	Initial Break Strength at 24 Hours, %			
	Trial 1	Trial 2	Trial 3	Average
Air flush	71	82	89	81
10% CO_2	67	86	89	81
10 ppm ethylene	57	61	58	59
10 ppm ethylene + 10% CO_2	67	76	70	71
1 ppm ethylene	76	64	81	74
1 ppm ethylene + 10% CO_2	73	66	75	71

a. Explants treated as shown for the first 6 hours after excision. After the treatment period all of the bottles were flushed with air and resealed and ethylene was added to the gas phase to give a final concentration of 10 ppm.

The data in Table 2 suggest that not only does ethylene accelerate aging, but that it may be a requirement for it. The idea in this experiment was to age explants in various concentrations of ethylene for longer periods than that used in the earlier experiments. The data show that the effect of high concentrations of ethylene on cell separation depended on low levels of ethylene during the aging period. The time period selected for an aging period was arbitrary and represents a compromise between an ethylene effect on the aging period and a second effect on inducing cell separation.

TABLE 2. REQUIREMENT FOR ETHYLENE DURING AGING[a/]

Ethylene Treatment, ppm		% Initial Break Strength
0 to 24 Hours	24 to 28 Hours	at 28 Hours ±SD
0.0	0.0	93±6
0.0	10.0	93±2
0.01	0.01	98±4
0.01	10.0	90±3
0.1	0.1	68±3
0.1	10.0	41±4

a. Explants were placed in a humidified gas stream containing various ethylene levels for 24 hours to accelerate aging. This was followed by a second treatment of 4 hours to induce cell separation.

IV. DISCUSSION

Stage 1 is defined as the period of time after excision IAA will prevent the loss of break strength. Operationally this is measured by injecting IAA into explants at fixed intervals and determining the time at which IAA is no longer capable of blocking the loss of break strength compared with an initial control. Basically the idea has been that stage 1 is concerned with the initiation of aging processes that lead to stage 2, the stage in which ethylene is capable of promoting cellulase synthesis. We had thought earlier that ethylene was without effect on stage 1 because it had no effect on abscission when applied during the first few hours after excision.[3] While the experimental observation was correct, and was confirmed by dela Fuente and Leopold[7] and data in Figure 2, the interpretation was wrong. It is now apparent that the correct interpretation is that ethylene does accelerate aging. This was shown by the experiments summarized in Figures 1, 2, and 3.

Figure 1 shows that the longer the explants were exposed to ethylene, the greater the subsequent loss of break strength. Had ethylene been without effect during the first few hours after excision, the initial part of the curve would have been horizontal instead of the more or less straight line actually observed.

Figure 2 shows that the aging effect of initial ethylene applications is realized only when it is followed by a second application of ethylene. In other words, the ability of ethylene to reduce break strength during stage 2 is greater when the explants are aged with ethylene during stage 1.

The aging effect of ethylene was also shown by measuring the ability of ethylene to shorten stage 1, indicated by the inhibiting action of IAA on break strength. Figure 3 shows that ethylene shortened stage 1 because auxin lost its abscission-retarding effect sooner in ethylene than in air.

The three experiments used to characterize a typical ethylene response are: (i) a dose-response curve, (ii) competitive inhibition with CO_2, and (iii) the relative activities of ethylene analogues such as propylene, acetylene, and CO. The results of the first two kinds of experiments are shown in Figure 4 and Table 1.

The dose-response curve shown in Figure 4 is similar to that observed for other ethylene effects such as growth inhibition: half-maximal activity in the neighborhood of 0.1 ppm and saturation at 10 ppm.

Competitive inhibitor experiments with CO_2 were only partially successful. Table 1 shows that 10% CO_2 partially blocked the effect of 10 ppm ethylene but failed to reverse the effect of 1 ppm ethylene. These results are unexpected, and no explanation for the discrepancy is obvious. If anything, CO_2 should have failed to block the 10 ppm ethylene treatment and blocked the lower (1 ppm) treatment.

The preceding experiments are concerned with demonstrating an accelerating effect of ethylene on aging. The next question that arises is whether ethylene is required for aging. The experiment shown in Table 2 was designed to measure whether aging could take place in the absence of ethylene or, more realistically, in the presence of very low ethylene concentrations achieved by flushing air past the explants. The data shown do not prove, but rather are consistent with, the idea that explants will not age in the absence of ethylene. A 4-hour 10-ppm treatment was effective only when explants were exposed to 0.1 ppm the preceding 24 hours.

In conclusion, the data presented here suggest that ethylene plays a dual role in abscission of petiole explants. First, it promotes or is actually required for aging, and secondly, it induces the enzymes required for cell separation.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
ABSCISSION: THE PHYTOGERONTOLOGICAL EFFECTS OF ETHYLENE

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Frederick B. Abeles Gerald R. Leather
Lyle E. Craker

6. REPORT DATE
June 1970

7a. TOTAL NO. OF PAGES
17

7b. NO. OF REFS
14

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 1B562602AD04

c. Task-Work Unit 02-001

d. DD 1498 Agency Access. DA OL 0003

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 610

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
CMs 6683, AMXFD-AE-T 49830

10. DISTRIBUTION STATEMENT
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Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES
Plant Physiology Division

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick, Frederick, Md., 21701

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14. Key Words

Abscission
Ethylene
Aging

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Unclassified
Security Classification